Identification and Mapping of Russian Thistle (Salsola tragus) and its Types

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Russian thistle, *Salsola tragus* L., is an annual tumbleweed infesting 41.3 million hectares in the western United States. Originally from southwest Asia, it was accidentally introduced into South Dakota in the 1870's. Since then, it has spread over most of the central and western United States and southern Canada. Russian thistle is now found in much of California, especially in the drier lands of the southern and western San Joaquin Valley. It causes considerable damage as a roadside weed, as a weed in certain crops and by fouling canals. It also acts as a reservoir for important insect pests, including the beet leafhopper. The leafhopper transmits curly top virus, a virus that infects several hundred varieties of ornamental and commercial crops, especially sugar beets, tomatoes, and melons in California. The problem has led to a control program costing approximately \$1.2 million per year, and requiring extensive insecticide treatments of the leafhoppers in Russian thistle stands.

An Eriophyid mite is currently undergoing host safety testing as a possible biological control agent for Russian thistle. This is the first new effort against the weed since the release of two Coleophorid moths in the early 1970's. Both species can be found in most localities that have Russian thistle, but they have not had much impact on the plant. When the mite or other agents are ready for release, their impacts will need to be evaluated in order to measure the performance of the project. Work this past season prepares for managing the future release of natural enemies and documenting impacts on the Russian thistle population in California by; 1) identifying potential new species that have until recently all been identified as *S. tragus;* and, 2) mapping the distributions and density of Russian thistle and, if possible, its various types.

The plant commonly known as Russian thistle is actually a grouping rather than a uniform species. Earlier work had shown that there are at least two types of Russian thistle distinguishable by enzyme and DNA differences (Ryan and Ayres 2000). They are referred to as Type A and Type B, with Type A being the traditionally recognized S. tragus. However, there were questions as to whether the two types could be distinguished morphologically. Dr. Fred Hrusa, Senior Plant Taxonomist, California Department of Food and Agriculture, was working on a limited set of specimens from a common garden found several promising morphological characters of these two main types. This year's effort was intended to verify their usefulness in the field. It also provided an opportunity to gather more diverse specimens for the continuing search for useful characters. This was especially important for Type B, which was represented by only a few populations in the common garden material. A secondary objective was to begin accurate mapping of the weed, particularly any variants. Identifying cryptic species is important because biological control agents are often highly specialized, and an unrecognized species could possibly be unaffected by biocontrol agents, allowing it to simply replace the weed controlled by the introduced agents. Mapping is important to target the sites of releases, identify research locations, and follow changes in the weed population in order to document impact.

Isoenzyme assays were used to verify field (or herbarium) identifications of the type or species of field-collected specimens. These assays were based upon aspartate amino transferase (AAT) and 6-phosphogluconate dehydrogenase (6-PGDh) and give clear separations of Types A and B, providing evidence for the possible existence of cryptic species. For each plant collected and identified in the field, a herbarium specimen was prepared and tagged for later morphological study and a matching tagged sample was run through the isoenzyme assay.

Some plants were also run through RAPD DNA analysis in order to begin a more detailed study of the relationships of the types gathered during the course of the survey.

Because the morphological character sets need to be as broadly useful as possible, specimens were collected at many sites within the Central Valley and into the Mojave Desert. A broad survey also meets the need to map the distribution and density of the types about the state. A total of 89 sites were visited, of which 12 sites were in the Mojave. Generally, a site was selected to be at least 15 miles from a previous collection site. Areas where Type B had previously been reported were targeted for visits, as it was desirable to ensure that both types were well represented in the collections, and Type B appeared to be somewhat uncommon in earlier work. If possible, sites were selected that had at least several dozen plants, but it was common to cover many miles in the agricultural areas of the Valley without seeing any Russian thistle. In these cases, sites with even a single plant were visited. Unfortunately, there was no method to efficiently record the many locations where no Russian thistle was seen. Sites were scored for the distribution of plants relative to the roadside (roadside only, away from roadside only, or both), a visual estimate of the size of the patch, and a visual estimate of the relative abundance of the types. In the latter half of the survey, after enough Type B had been collected for morphological and isozyme studies and field staff could recognize Type B in the field, a point-line transect through many of the stands, noting the type or absence of Russian thistle at two meter intervals.

Field observations were made on 220 plants, of which 150 were collected for the herbarium and for isoenzyme assays. Plants were given a field identification as to Type or species (in the case of *S. paulsenii*), scored for the characters that promised to best separate Type A and B (length of anthers, subjective estimate of abundance of winged seeds, form of minor lobes on winged seeds, maximum diameter of the winged seeds, the placement of winged seeds on the plant, and hairs on stems), and for height and two width measurements, to give a rough measure of volume. Typically, two specimens were collected for each Type present. Plants were chosen to represent the range of variation at the site. If there was a wide range of variation of a Type at a site, more than two specimens were collected. At 17 sites, only Type A plants were found and they seemed to fall within the range of variation that had already been collected in nearby locations, so no plants were collected.

Type B was found to be generally uniform in its characters, and scientist learned to recognize it routinely and easily in the field (Table 1). On the other hand, Type A was highly variable and may grade into another type, Type C, and possibly into a form of *S. paulsenii* (the lax form). It may prove difficult to consistently identify these latter groups, at least in the field.

Many of the important field characters involve the flowers and seeds. The winged seeds of Russian thistle are unusual among plants and an explanation may be helpful. The winged seeds look like flowers on casual observation. The true flower is nondescript and is chiefly marked by the expansion of the anthers. After flowering, the calyx remains wrapped about the developing seed. An auxiliary lobe on the sepal may develop into a "wing" that gives the appearance of a petal on a flower. Whether the lobes develop depends on the Type, on the individual plant, and on the location of the flower on the plant. Seeds can form without having any development of the sepal lobes. This is common in Type A. In seeds that do develop wings, not every sepal of the calyx develops equally. Three sepals will develop larger, sub-equal "major" lobes. Alternating with them, the lobes of the remaining two sepals will expand to a varying extent, depending on type and species. They are always clearly smaller than the major lobes, sometimes little more that triangular nubs. Type B has exceptionally large and obvious minor lobes.

In the field, the most useful characters for verifying Type B were the length of the anther and the form of the minor seed lobes. In Type B, the minor seed lobes are rounded, large, and lay on top of the major seed lobes, such that they are easily visible in the field, even without a hand lens. The anthers, when they are present, are small, 0.5 to 0.7 millimeters (mm). Other useful field characters were that Type B plants always have seeds with expanded wings (in the latter part of the season), the winged seeds appear both low and high on the plant and the internodes between bracts are generally long relative to the bract length. These latter characters give the plant an open, white-spangled appearance that is generally easily recognizable even from several meters. All these characters are in general distinctive from Type A and the other possible forms. In Type A, the minor lobes are much smaller than the major lobes and are generally hidden between or beneath them, such that they are hard to find in the field. They may be small triangular nubs, or oblong with slightly expanded tips.

Type A is highly variable in many of its characters. Some plants never develop any winged seeds or only very few, even at the very end of the season (November), though such plants are often quite large, over a meter in every dimension. This in itself will rule out a plant as Type B, as Type B always has winged seeds, at least after July. Type A plants without winged seeds often have an abundance of anthers, even late into the season. Seeds are present, even though they do not have wings. Plants without winged seeds generally have long internodes between bracts, these being congested only near the branch tips. Such plants appear relatively open and often have a lacy, soft appearance. At the other end of the scale, many Type A plants can have an abundance of winged seeds. In the latter plants, the winged seeds generally occur in the outer, upper parts of the plant, towards the outer half of the branches. This is unlike Type B, where the winged seeds also occur toward the base of the branches and plant. In Type A plants with abundant winged seeds, the internodes between bracts are short and the winged seeds arise close together, giving the plant a dense, bristly, compact appearance, again unlike Type B. The full range of variability of Type A can occur at a single site.

The only character that does not appear to vary much for Type A is the length of the anther, at approximately 1.1 to 1.3 mm, as opposed to 0.5 to 0.7 mm for Type B. If anthers are available, they alone can distinguish Type B from other types and species.

Winged seed diameter is not a good character for distinguishing a plant, at least in the field. Type B plants were consistent with diameters running five to eight millimeters. Type A plants were highly variable. Some plants with winged seeds had diameters in the two to three mm ranges, but plants with a range of four to seven mm were more common.

Stem hairs were occasionally useful. Type B appears to be always glabrous. Type A usually has stem hairs of some form, but glabrous individuals are common. In addition, in the field it is often difficult to clearly see far back on a stem or major branch.

Field identification of the different types generally agreed well with isozyme identifications, especially after a little experience with Types A and B in the field (Table 1). Isozyme BB plants were consistently identified as Type B in the field. Isozyme AA plants were nearly always identified as Type A (the two isozymes, AAT and 6-PGDh, cannot distinguish *S. paulsenii* from Type A and therefore the three *S. paulsenii* among the AA group in (Table 1) are not misidentified). Beyond Type B lays a range of other morphologic and isozyme types (Table 1) that are beginning to show some consistent relation to one another, although more work with the putative Type C and the forms of *S. paulsenii* is needed. Type C had been noted occasionally in earlier work and continued to appear in the current collections. Of the 21-isozyme California plants, 10 were identified in the field as Type A, but six of those 10 plants were from the first collecting bout, when we were first becoming familiar with variation in the

field. That so many of the California plants caused difficulties in their identification, especially later in the collecting period, indicates that the field biologist was becoming aware of differences that distinguish this type. Many isozyme California group plants had minor wing lobes that were somewhat expanded beyond that typical for Type A, or had non Type B winged seeds that nonetheless occurred both low and high on the plant, or they were intermediate in the length of the anther. More collection and study of isoenzyme Type C plants may well lead to morphological characters than can distinguish this type.

Table 1: Comparison of Isoenzyme Identifications Versus Field Identifications of Individual Plants

Isozyme ID's: AAT + 6-PGDH	Number of Plants	Field ID's and Number of Plants			
7	1 1011100	Α	В	P	Other
AA ¹⁾	73	66		3	B? ²⁾ = 1
					A? = 3
BB	48		47		B? = 1
AB	3	2			B? = 1
CA	21	10			A? = 3
					A $mwEx = 2$
					A Low = 2
					A or $P = 1$
					B? = 3
A"A	3				A Low $= 3$
AA'	3	2			B? = 1
AB'	1	1			
AB*	2	2			
C'A	1			1	

¹⁾ XX = the type identification provided by AAT plus the type identification provided by 6-PGDh. For example, AA means isozyme AAT identified the plant as Type A and 6-PGDh also identified it as Type A. A = Type A, B = Type B, C = Type C: A', A'', B', B*, and C' were possible variants from the main types.

Mapping of the site collections confirms general impressions of Russian thistle as a roadside weed in most of the Central Valley and western Mojave. In heavily agricultural areas, Russian thistle was actually uncommon, even along the roads. It was generally common only along some of the major highways and railroads, and in the peripheries of towns. Usually it is found away from the roadside only near towns, in vacant lots and similar locations that receive occasional (not regular) disturbance. The only area where Russian thistle was growing abundantly is in the general landscape was in the area of the Elk Hills, in southwestern San Joaquin Valley. The search only went as far north as Marysville, but Russian thistle still occurred there. Type A was found throughout the survey area. Type B was only found south of Tracy, and not at all in the Mojave. An earlier survey had found Type B as far north as Marysville, but the current search in the same areas found none. The precise locations where Type B had been found previously were not visited during this survey.

Type B is generally not as common as Type A, but it seems to have a population concentration around the town of South Dos Palos and along Highway 133 south into Mendota. The two

²⁾ B? = The plant seemed most likely to be a B but the identification was difficult: A? is similar; P = S. paulsenii; A mwEx = a note was made that minor wing was somewhat expanded, intermediate between A and B; A Low = plants seemed to have A-type winged seeds (relatively small minor lobes) but they arose LOW on the plant, opposite to the usual pattern in Type A.

types can occur in pure or mixed stands. In mixed stands, Type A is generally more abundant. Line transects were performed at 26 collection sites and mixed populations were found at three of these sites. The average percent cover of Type B at the three sites was 3.2 percent, while it was 38.0 percent for Type A. While this is a very small sample, the results are consistent with subjective impressions. With regard to the frequency of finding sites with the different types, of the 89 sites visited in the survey, 49 were in the San Joaquin Valley south of Tracy. Of those 49 sites, 23 had Type A only (sometimes possibly mixed with Type C), 14 had Type B only (sometimes possibly mixed with Type C), and nine had mixed stands. Of the remaining three sites, one had *S. paulsenii* only and the other two possibly had only Type C. The results from these 49 sites almost certainly overstate the relative frequency of Type B because Type B was a conscious target of the survey. Still, Type B is not rare within its range, though not as common as Type A.



